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Introduction:

This project is intended to explore the role of polycomb group protein EZH2 and its binding partner EED in metastatic prostate cancer. Both proteins will be introduced and overexpressed independently overexpressed in prostate cancer xenograft models by lentiviral transduction. Concordantly, the effects of reduced EZH2 or EED expression will be evaluated by RNAi technology. The growth and progression of the tumors will be monitored by optical imaging. The hypothesis is that EZH2 serves as a master regulator protein in prostate cancer metastasis; therefore, disruption of the balance between EZH2 and EED will result in a more aggressive disease phenotype. Exploration of the relationship between EZH2 levels and kinetics of dissemination of cancer cells will result in a better understanding of disease progression and appropriate treatment approaches.

Body:

Aim 1: Generation of lentiviral vectors that overexpress EZH2 or EED and that suppress expression by RNA interference (RNAi).

The lentivirus vector to deliver and overexpress EZH2 (pCCL-CMV-EZH2-IRES-EGFP) and the control vector (pCCL-CMV-IRES-EGFP) were constructed and produced. The use of EGFP, a fluorescence gene, rather than the originally proposed luciferase allows us to monitor vector transfection and transduction, and to deduce the expression levels of EZH2 by fluorescence microscopy, making production and evaluation of the vector more rapid and efficient. The luciferase gene to facilitate optical imaging of tumor growth and progression was introduced into cells by co-infection with a separate lentivirus (pCCL-CMV-RL).

pCCL-CMV-EZH2-IRES-EGFP was used to transduce 293T cells, which were then used to screen four unique siRNA sequences. Plasmids encoding the prospective siRNAs were transfected into the EZH2 overexpressing cells, which were then harvested at either 48 or 72 hours. Knockdown of lentiviral EZH2 expression was evaluated by western blot (Figure 1). Transducing cells with the EZH2 lentivirus proved more efficient and provided more reliable results than the original cotransfections (data not shown). Based on these results, siRNA 2.1 was selected for use in the xenograft model experiments. siRNA2.1 is currently being cloned into the lentiviral vector for production of the siRNA lentivirus.

Design and production of the EED lentivirus and EED specific siRNA are ongoing at this time.

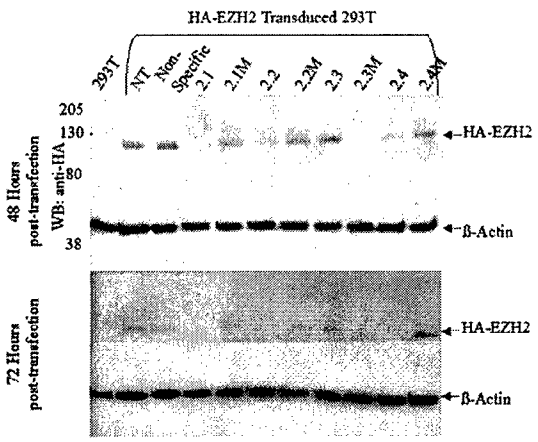

Figure 1: Screening of prospective siRNA sequences.
Four unique siRNA sequences were transfected into cells overexpressing EZH2. Cells were harvested and assayed for EZH2 levels at 48 and 72 hours post transduction. NT: Not Transfected with siRNA. Non-Specific: siRNA specific for luciferase. 2.1, 2.2, 2.3, and 2.4 are unique siRNA target sequences. 2.1M, 2.2M, 2.3M, and 2.4M are modified versions of the parent sequence to test for increased effectivity.

Aim 2: Monitor the effects of genetic modifications of PcG genes on metastasis of prostate xenograft by optical imaging.

Prostate cancer xenograft model LAPC9 cells were marked with the pCCL-CMV-RL lentivirus and either pCCL-CMV-EZH2-IRES-EGFP (EZH2 tumors) or pCCL-CMV-IRES-EGFP (control tumors) and implanted in SCID mice. Beginning two weeks after implantation, the mice were imaged to monitor tumor growth and the tumors themselves were measured. In both optical signal (Figure 2) and physical tumor size (data not shown), the EZH2 group showed an astounding growth advantage over the control group. Therefore, EZH2 overexpression appeared to confer an initial growth advantage in the LAPC9 xenograft model. By week 4, however, the initial advantage conferred by EZH2 overexpression had diminished to such a degree that the control tumors were equal to the EZH2 tumors in both optical signal and size. At the study endpoint (week 7), tumors were removed and analyzed for EZH2 expression by western blot. Interestingly, the EZH2 marked tumors showed no detectable EZH2 expression. This decline is possibly due to silencing of the integrated lentiviral genome or a decrease in CMV promoter activity die to conditions within the tumor. However, the drop in EZH2 expression may correlate with and be responsible for the deceleration in EZH2 tumor growth observed by week 4 of the study.

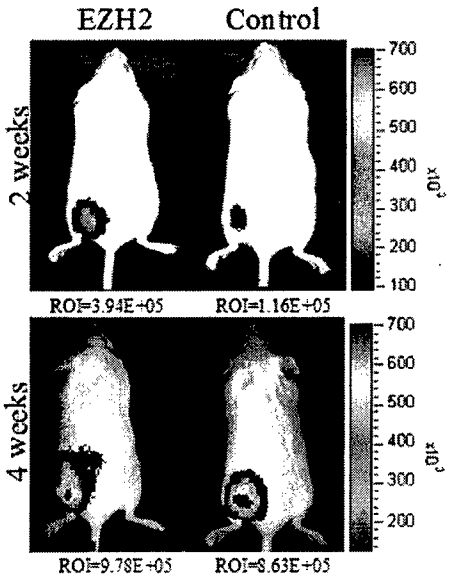

Figure 2: Side-by-Side Comparison of EZH2 versus Control Optical Signal at 2 and 4 Weeks.
Animals were imaged for luciferase signal as a means of tracking tumor growth and progression. At week 2 (top), animals with EZH2 overexpressing tumors (left) clearly demonstrate higher signal than animals with Control tumors (right). By week 4 (bottom), however, this inequity has resolved due to slower growth by the EZH2 tumors.

To attain more control over the expression of EZH2 in the tumor environment, we initiated the construction of a Tet regulated lentiviral expression system. The use of the TetON system will allow EZH2 expression only in the presence of the inducer doxycycline. This system was demonstrated to be highly titratable and controlled in preliminary experiments (Figure 3). Use of the TetON lentivirus system to manage the level and length of EZH2 expression will permit more control over the stimulus provided to the tumor. In addition, we will be able to more conclusively determine if decline in EZH2 expression was truly responsible for the decrease in tumor growth rate.

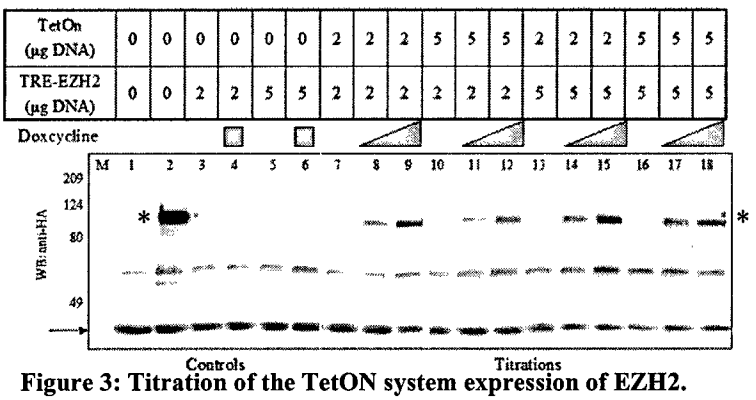

Figure 3: Titration of the TetON system expression of EZH2.
293 cells were cotransduced with the elements of the TetON system in varying ratios and tested for induction with increasing amounts of Doxycylcine. Lanes 1-6 are controls. Lanes 7-18 are titrations of the TetON system. Asterisk denotes EZH2 band. Arrow denotes β-actin control for equal loading. A middle non-specific band was detected.

Key Research Accomplishments:

- Construction and generation of pCCL-CMV-EZH2-IRES-EGFP lentiviral vector.
- Successful screening of prospective EZH2 siRNA sequences. Identification of siRNA2.1 as top inducer of RNAi effect.
- Seminal study of effects of EZH2 overexpression in prostate cancer xenograft model, monitored by optical imaging. Preliminary results indicate a growth advantage conferred by EZH2 overexpression.
- Initiation of construction on a TetON lentiviral expression system for EZH2. System is highly regulated and titratable.

Reportable Outcomes:

Abstracts:

Breanne D. White and Lily Wu. Characterizing the effects of EZH2-overexpression in prostate cancer progression using in vivo optical imaging. AACR Annual Meeting 04/2005

Funding:

Breanne White secured the Predoctoral Cell and Molecular Biology Training Program, UCLA. (07/2004)

Conclusions:

We discovered that EZH2 overexpression in a prostate tumor induced tumor cell proliferation. However, long-term effect of EZH2 cannot be investigated as lost of its expression was selected for in the tumor. Thus, we have created a titratable, tetracycline regulated system to express this important regulatory gene. The regulated expression will enable us to better define the functional role of EZH2 in tumor progression. The knockdown study using stable siRNA expression system is proceeding without difficult. In the next 6 months, we would be able to accomplish all the proposed tasks of the project. Our intention is to extend this conceptual idea to a comprehensive study of this very interesting polycomb gene, EZH2.